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Stable carbon isotope ratio for sugar, amino acid, and caffeine by liquid chromatography/isotope ratio mass spectrometry

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Abstract

In 2004, liquid chromatography coupled with isotope ratio mass spectrometry (LC/IRMS) has been developed. Today, LC/IRMS system has been used for various compounds. Stable isotope analysis has proved to be a powerful tool for source apportionment of various compounds. We analysed $\delta^{13}C$ of 6 sugar, 10 amino acid and 2 caffeine samples using direct injection mode and online LC/IRMS mode. In this study, the ion chromatography system was coupled with IRMS instrument.

On direct injection mode, the standard deviation for all samples were <0.26‰. The difference between EA/IRMS and direct injection mode was 0.41‰ (sugar), 2.01‰ (amino acid), 5.09‰ (caffeine, Wako Pure Chemical Industries), and 7.41‰ (caffeine, IAEA). The differences for amino acid and caffeine samples were bigger than sugar samples. The reason of the difference is still unclear.

In the case of online LC/IRMS mode, the differences between the EA/IRMS and online LC/IRMS without phosphoric acid for amino acid were from 2.54‰ to 9.93‰. In contrast, when 1% phosphoric acid was added, the differences between the EA/IRMS and online LC/IRMS was ranged from 0.09‰ to 2.09‰. The eluent for LC was 5mM sodium tetraborate decahydrate (pH=9.51). When the 1% phosphoric acid was not added, the oxidation potential of sodium peroxodisulfate was decreasing. Thus, when the eluent of alkalinity eluent was used, it is necessary to add phosphoric acid. However, the difference of 2 caffeine samples between EA/IRMS and online LC/IRMS without phosphoric acid and with phosphoric acid were 5.30‰ and 5.82‰, and 5.68‰ to 7.32‰, respectively. For caffeine samples, the $\delta^{13}C$ did not change with or without phosphoric acid. Other reasons were considered e.g. incomplete combustion.

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Keywords: LC/IRMS; CSIA; carbon isotope, amino acid, caffeine

1. Introduction

In 2004, liquid chromatography coupled with isotope ratio mass spectrometry (LC/IRMS) has been developed[1], which allows the measurement of stable carbon isotope ($\delta^{13}C$) composition of highly polar and non-volatile analyses without the need for derivatization. The interface is based on a wet oxidation of organic compounds in aqueous solution to produce CO_2 gas, and separate CO_2 from the liquid phase. Today, LC/IRMS system has been used for various compounds e.g., sugar[1-3], amino acid[4,5], alcohol[6,7] and pesticide[8]. Stable isotope analysis has proved to be a powerful tool for source apportionment of various compounds. However, the research of precision and accuracy for LC/IRMS were very limited until now. In addition, the potential effect of including nitrogen has not been investigated.

We analyzed $\delta^{13}C$ of sugars, amino acid and caffeine samples using direct injection mode and online LC/IRMS mode.

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Firstly, accuracy and precision in this method were investigated in the concentration range of L-alanine and L-histidine from 200 to 6,500ngC and 110 to 7,000ngC, respectively. Secondly, we measured sugar, amino acid and caffeine samples using direct injection mode. We compared direct injection mode with EA/IRMS. Finally, we measured amino acid and caffeine using online LC/IRMS mode. This study presents a new application using common anion exchange column to the determination of $\delta^{13}C$ of amino acid and caffeine samples.

2. Method

Analytical samples of D(+)-Glucose, D(+)-Xylose, D(+)-Galactose, D(+)-Mannose, Sucrose, D(-)-Fructose, L-Alanine, L-Histidine, L-Leucine, L-Valine, Glycine and Caffeine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan; purity of at least 98%). L-Alanine and L-Histidine were purchased from Shoko Co., Ltd. (Tokyo, Japan). Sodium peroxodisulfate (99%) and phosphoric acid (99%) were purchased from Sigma-Aldrich (Tokyo, Japan) and Kanto Chemical CO., Inc. (Tokyo, Japan). In order to avoid degassing of CO_2, all solutions were continuously sparged with helium.

The stable carbon isotope ratios of the reagents were determined by LC/IRMS. The ion chromatography system (ICS1000; Dionex, Sunnyvale, CA, USA) was coupled with an IRMS instrument (Isoprime Ltd., Cheadle Hulme, UK). An anion exchange column (4.0 × 200mm, IonPac AS12A with a 4.0 × 50mm; AG12A guard column; Dionex, Camberlyey, UK) was used. The eluent was 5mM Sodium tetraborate decahydrate with a flow rate of 0.3-0.35mL min^{-1}. The volume injected is 20μL (direct injection mode) and 25μL (online LC/IRMS mode).

The carbon stable isotope composition, expressed in the delta (δ) notation in permil (‰) units, was calculated as follows:

$$\delta^{13}C[‰] = \left(\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{std}} - 1 \right) \times 1000$$

where $^{13}C/^{12}C_{sample}$ and $^{13}C/^{12}C_{std}$ are the atomic ratios of ^{13}C to ^{12}C in the sample and in the Vienna Pee Dee Belemnite (VPDB) standard, respectively. All samples were measured in triplicate. At the beginning of each run, two CO_2 gas pulses of a laboratory standard gas ($\delta^{13}C$ = –33.36‰) were introduced. Reference materials IAEA-CH-3 (cellulose, $\delta^{13}C$ = –24.72‰), IAEA-600 (caffeine, $\delta^{13}C$ = –27.77‰), and USGS24 (graphite, $\delta^{13}C$ = –16.05‰) were used.

Elemental analyzer (EuroVector, Isoprime Ltd., Cheadle Hulme, UK) coupled with the same IRMS system was used to determine the $\delta^{13}C$ values of the analytical standards and bulk isotope ratios of samples. All data were averaged, and a two-point linear calibration was carried out for $\delta^{13}C$ against standards. International isotopic standards including IAEA-CH-3 (cellulose), IAEA-600 (caffeine), and USGS24 (graphite) were also used.

3. Results

Accuracy and precision: The $\delta^{13}C$ values of L-alanine and L-histidine were determined in a concentration range from 200 to 6,500 ngC and from 110 to 7,000 ngC using a 20 μL loop injection, respectively. The $\delta^{13}C$ yielded values for L-alanine and L-histidine from –21.68‰ to –19.43‰ (S.D.; <0.30‰) and from –12.91‰ to –11.94‰ (S.D.; <0.40‰), respectively. The standard deviations during the concentration range were very reasonable. However, for L-alanine, the $\delta^{13}C$ value was 1‰ lighter for concentrations higher than 4,000 ngC compared to the EA/IRMS. For L-hidtidine, the $\delta^{13}C$ value was approximately 3‰ lighter for all concentrations. There was good linear relationship between the total peak area and concentration in alanine (R^2=1.00). However, for L-histidine, there was not good linear relationship, and, for concentrations higher than 4,500ngC the peak area was not increasing. The reason for L-histidine was not perfectly combustion..

Direct injection mode results: The standard deviation for sugar and amino acid, caffeine samples were <0.26‰. The relationship between EA/IRMS and direct injection mode with or without phosphoric acid are shown in Fig. 1: (a) 6 sugar samples, and (b) 11 amino acid samples. The determination coefficient of both sugar and amino acid were approximately 1.00 with or without phosphoric acid. For sugar samples, the difference between EA/IRMS and direct injection mode was 0.41‰ (intercept) with phosphoric acid. However, the difference was 2.01‰ in amino acid. In addition, $\delta^{13}C$ values of two caffeine samples (Wako Pure Chemical Industries and IAEA) were lighter than EA/IRMS, from 5.09‰ to 7.41‰. The sugar samples in this study do not have nitrogen. In contrast, amino acid and caffeine samples have nitrogen concentrations ranging from 11.0 to 29.0 % of total molar weight. Thus, the difference reason may be considered incomplete combustion for nitrogen compounds.

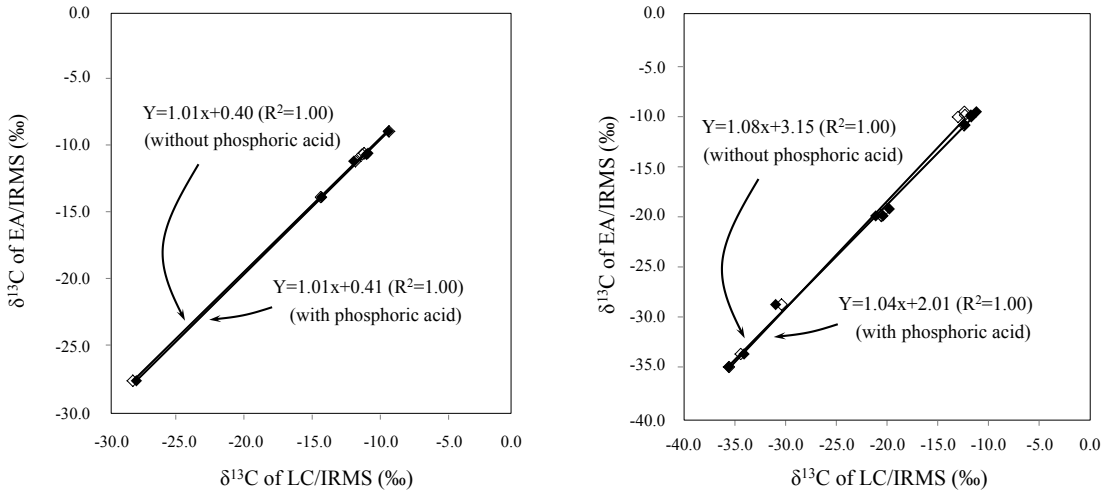

Fig. 1. The relationship between EA/IRMS and direct injection mode with phosphoric acid (solid symbols) or without phosphoric acid (open symbols) (a) 6 sugar samples; (b) 10 amino acid samples.

Online LC/IRMS mode results: In the case of online LC/IRMS mode, all results for amino acid samples with or without phosphoric acid were given in Fig 2. When 1% phosphoric acid was not added, the differences between the EA/IRMS value and online LC/IRMS value was from 2.54‰ to 9.93‰. In contrast, when 1% phosphoric acid was added, the difference between the EA/IRMS value and online LC/IRMS value was from 0.09‰ to 2.09‰. The eluent used for ICS1000 is 5mM sodium tetraborate decahydrate (pH=9.51). When the 1% phosphoric acid was not added, the oxidation potential of sodium peroxodisulfate was decreasing. Thus, when the eluent of alkalinity eluent was used, it is necessary to add phosphoric acid. However, the difference of 2 caffeine samples between EA/IRMS and online LC/IRMS without phosphoric acid and with phosphoric acid were 5.30‰ and 5.82‰, and 5.68‰ to 7.32‰, respectively. For caffeine samples, the $\delta^{13}C$ did not change with or without phosphoric acid. Thus, the difference reason may be considered incomplete combustion for nitrogen compounds.

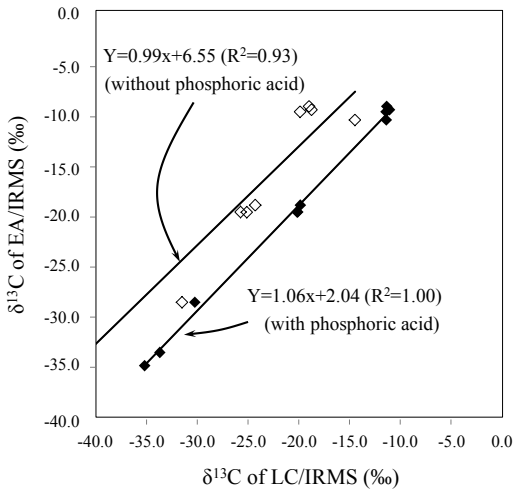

Fig. 2. The relationship between EA/IRMS and on-line LC/IRMS mode with phosphoric acid (solid symbols) or without phosphoric acid (open symbols)

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